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Protecting What Matters, Every Day

Managing Microbiological Risk with PCR and Automated Integrity Test Devices

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Microorganisms: The Good, The Bad and The Costly!!

- **Fermenting Strains**
  - Wines fermented with added yeast and/or wild yeast to provide uniqueness of product
- **Spoilage Organisms**
  - Organoleptic Impacts or Effects on Process
    - Off-flavours and sensory defects
    - Film formation or turbidity and sediment
    - Undesired fermentation
    - Decreased filterability and exploding products
- **Spoiled Product**
  - Product loss; costly recalls
  - Labor-intensive investigations after spoilage
    - Production interruptions and increased losses

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Managing the Risk of Spoilage

- Process control to help prevent contamination events
  - Mitigates risk
- Solutions for quality control:
  - Rapid detection and/or identification of microorganisms
    - PCR technology
  - Removal of microorganisms
    - Filtration with membrane filters
- **Pall solutions:**
  - Rapid detection and identification of microorganisms with GeneDisc
  - Ensure effective filtration through integrity testing with Compact Touch

Rapid Microbiological Detection and Identification with PCR Technology

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What is Polymerase Chain Reaction (PCR)?

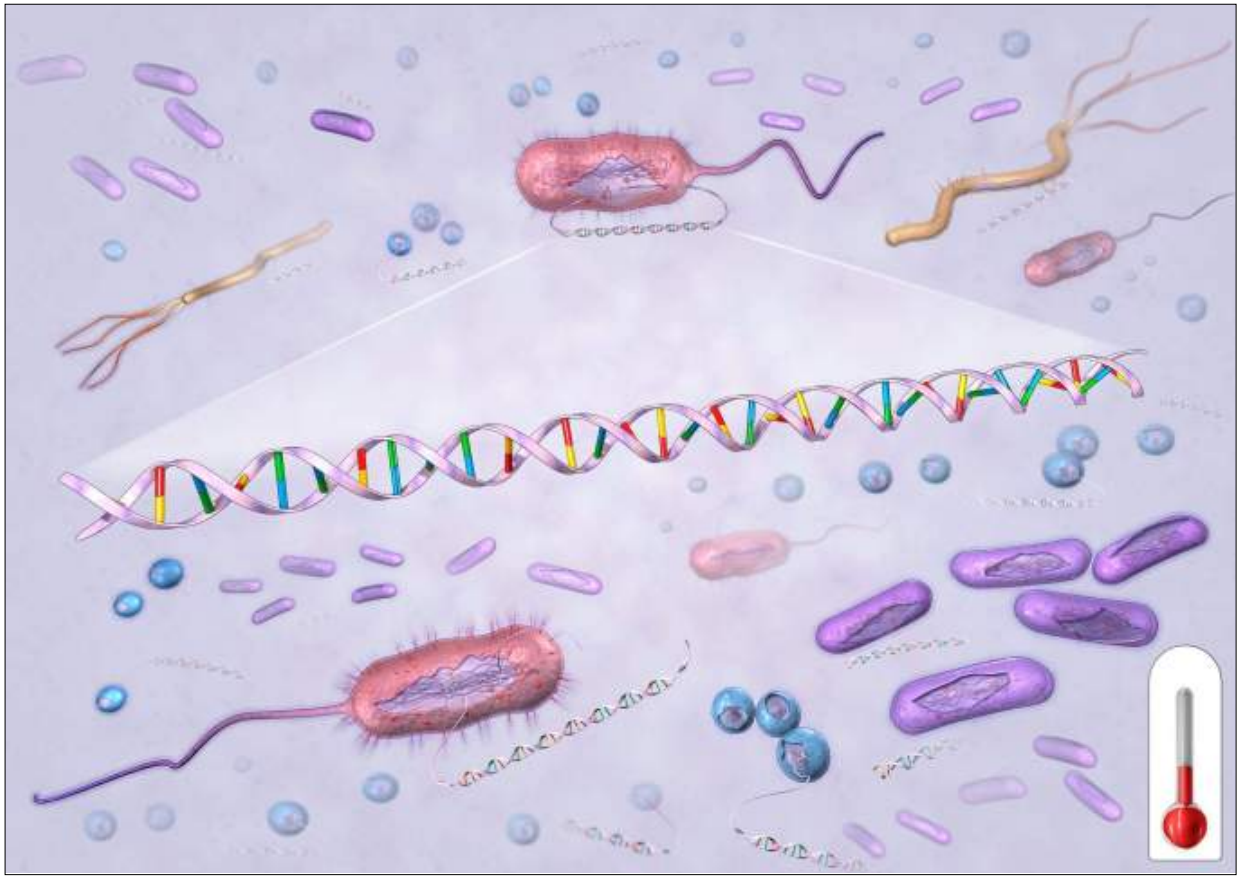
Method for amplifying specific DNA targets

Starting with one double strand of DNA

- 1 amplification cycle = 2 double strands
- 2 amplification cycles = 4 double strands
- 3 amplification cycles = 8 double strands
- 4 amplification cycles = 16 double strands
- 5 amplification cycles = 32 double strands
- 10 amplification cycles = 1,024 double strands
- 20 amplification cycles = 1,048,576 double strands
- 30 amplification cycles = 1,073,741,824 double strands
- 40 amplification cycles = 1,099,511,627,776 double strands

DNA Extraction

- DNA strand extracted from target microorganism



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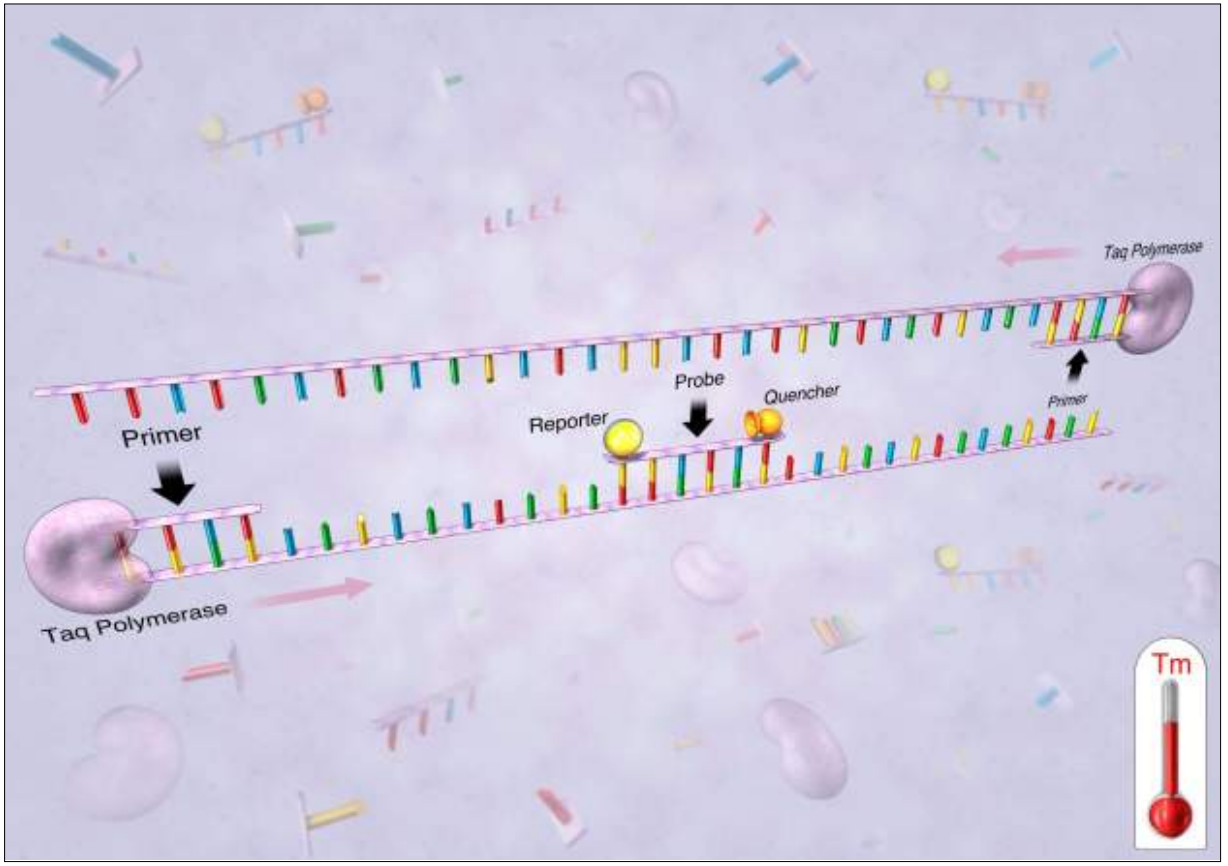
qPCR: DNA Amplification

- DNA unwound and double strand separated into two single strands

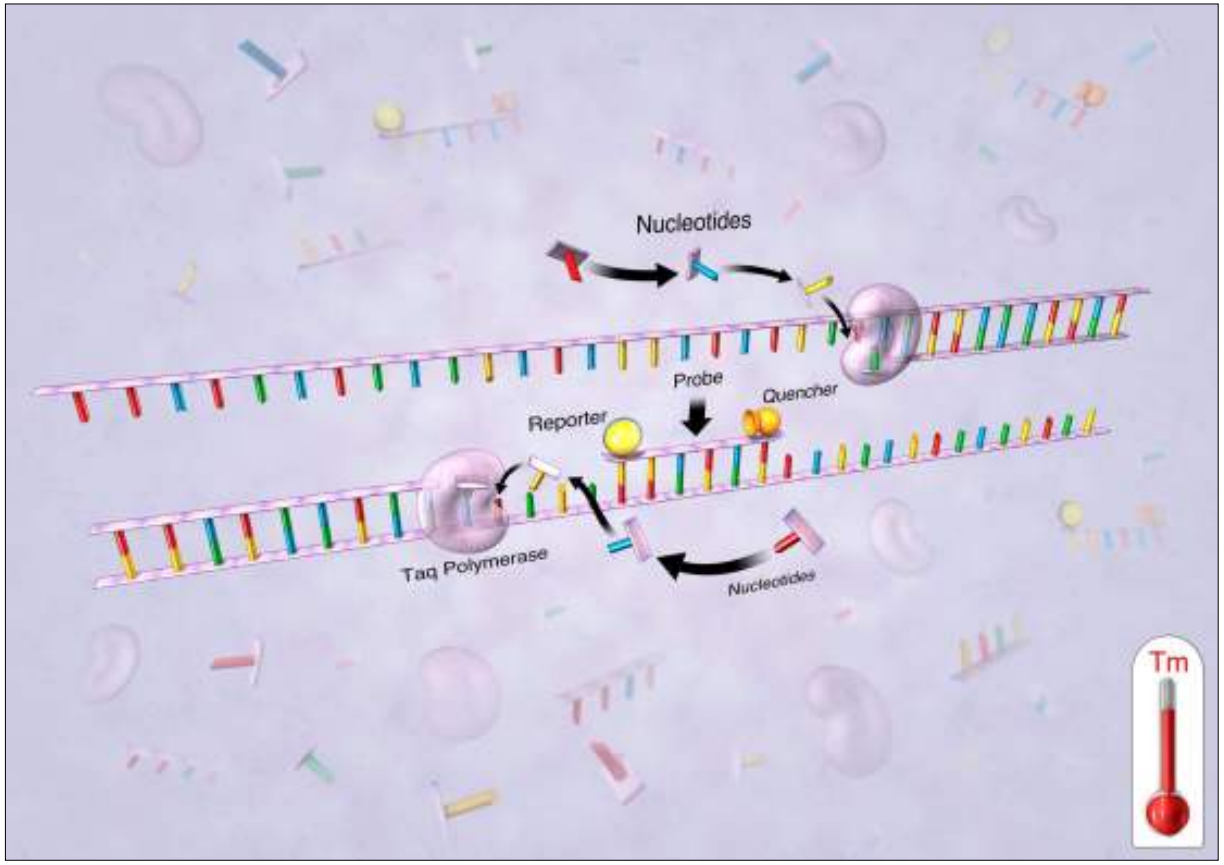
qPCR: DNA Amplification

- Hybridization of primers and probe onto two single strands



qPCR: DNA Amplification

- Synthesis of the complementary strand



qPCR: DNA Measurement

- As amplification occurs, reporter fluorescence is detected by emission of a specific light wavelength

qPCR: DNA Measurement

- The process is repeated for 25-45 cycles to amplify sufficient material for visualization

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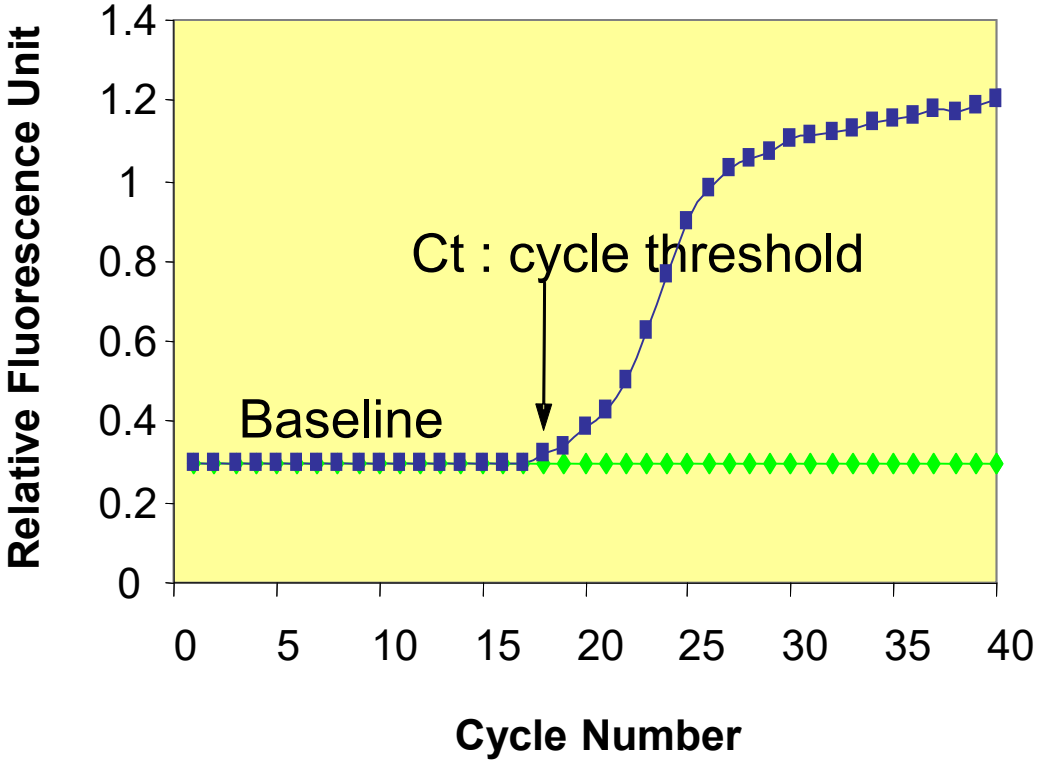
What is Quantitative or Real Time PCR?

- Measures amplification as it occurs
  - Reporter fluorescence is measured, data collected and plotted in real time
- When the target microorganism DNA is present, fluorescence increases with each cycle



Ct: Cycle at which the fluorescence from a sample crosses the threshold (fluorescence above background)

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Why Choose PCR Technology?

- **Faster time-to-results**
  - Speeds up batch/product release
  - Accelerated decision-making
  - Early preventative controls

- **Fast corrective actions implementation**
  - Rapid root cause analysis
  - Reduces negative financial impact of spoilage

- **Reduce Costs**
  - Product scrap or recall
  - Additional product processing related to product spoilage
  - Storage costs

GeneDisc® System

- Pall's GeneDisc system is an easy to use platform which allows accurate detection or quantification of microorganisms using the Real-Time Polymerase Chain Reaction (qPCR) method

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GeneDisc® System: From sample to result

- Microbial detection or quantification in three simple steps:
- **Step 1 -** Extract DNA
  - Universal extraction protocols
  - Frees DNA from microbial cells
- **Step 2 -** Fill the GeneDisc Plate
  - Ready-to-use consumable
  - Pre-loaded with all primers and probes in a unique, sealed plate
- **Step 3 -** Run the Real-Time PCR test
  - Using the GeneDisc Cycler

Flexible Protocols

- **Direct Monitoring Protocols**

  Get results in 2 hours

Assess Contamination in 2 hours

Cell concentration

Cell lysis

PCR analysis

Technical Information

Sensitivity	Filterable samples: As low as 1 cell/mL Unfilterable samples: As low as 85 cells/mL
Time to Results	Reduced to 2 hours
Plate Options	• Yeast Screening • Yeast ID for identification of the 12 major spoilage yeast genera and species simultaneously
Internal Positive Control	To ensure result accuracy, each sample analysis includes an internal positive control.

When quick results are your priority

Flexible Protocols

- **Enrichment Protocols**

  For greater sensitivity

Reach High Sensitivity

Enrichment

Cell lysis

PCR analysis

Technical Information

Sensitivity	Down to 1 cell / sample
Enrichment	As low as 28 hours
Time to Results	Enrichment time + 2 hours
Plate Options	• Yeast Screening • Yeast ID for identification of the 12 major spoilage yeast genera and species simultaneously
Internal Positive Control	To ensure PCR result accuracy, each sample analysis includes an internal positive control.

When **precise information** is your priority

Flexible Protocols

- ***Brettanomyces* Quantification**

Monitor *Brettanomyces* Level in 2 hours

Cell concentration

Cell lysis

PCR analysis

Technical Information

Quantified Targets	*Brettanomyces* spp. and *Brettanomyces bruxellensis*
Sample Types	Designed for wine process samples from grape must to bottling
Sensitivity	As low as 1 cell/mL
Quantification Range	As low as 1 to 100,000 cells/mL
Time to Results	Reduced to 2 hours
Detected Targets	Allows simultaneous detection of 10 additional spoilage yeast genera and species
Internal Positive Control	To ensure result accuracy, each sample analysis includes an internal positive control.

To preserve wine sensory characteristics

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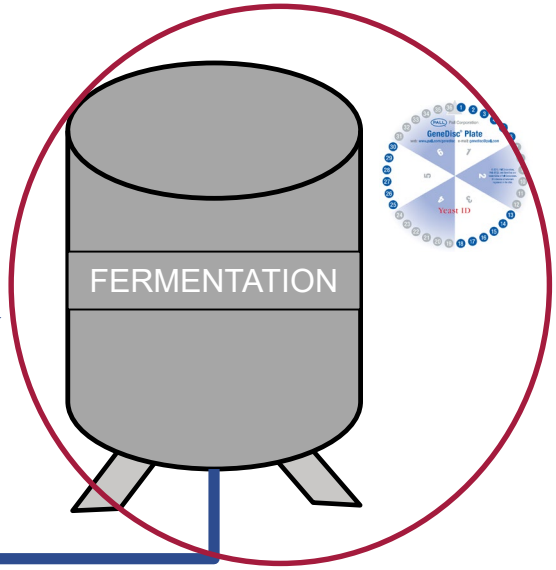
Example of Implementation in Winery

HARVEST

Monitor fermentation

Control *Brettanomyces* level during pre-fermentation and between fermentation steps

With Yeast ID



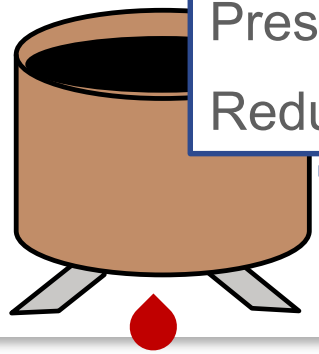
PRESS

AGING

BOTTLING

Preserve your wine sensory characteristics

Reduce product scrap/ unnecessary rework



FINISHING

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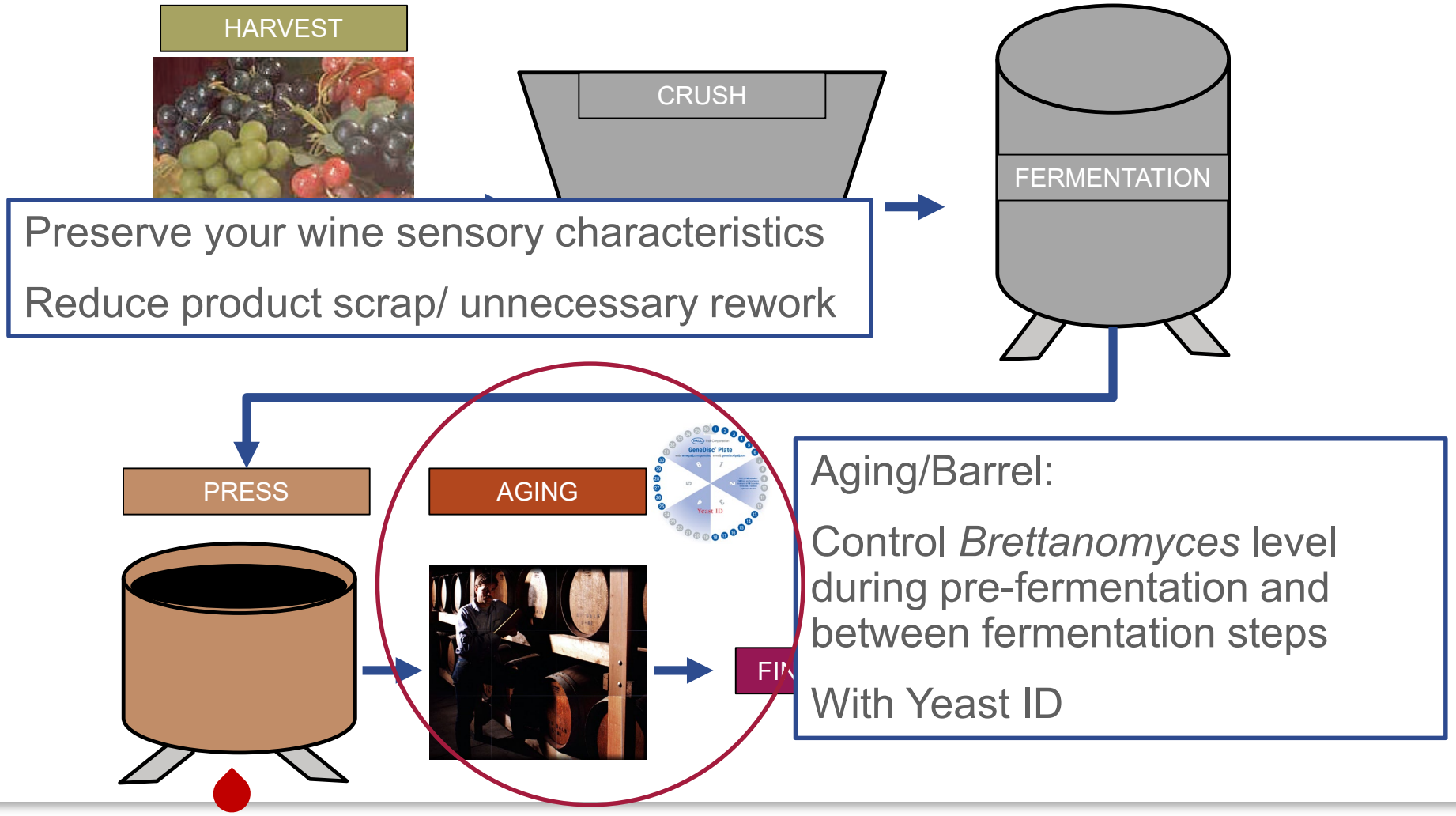
Example of Implementation in Winery



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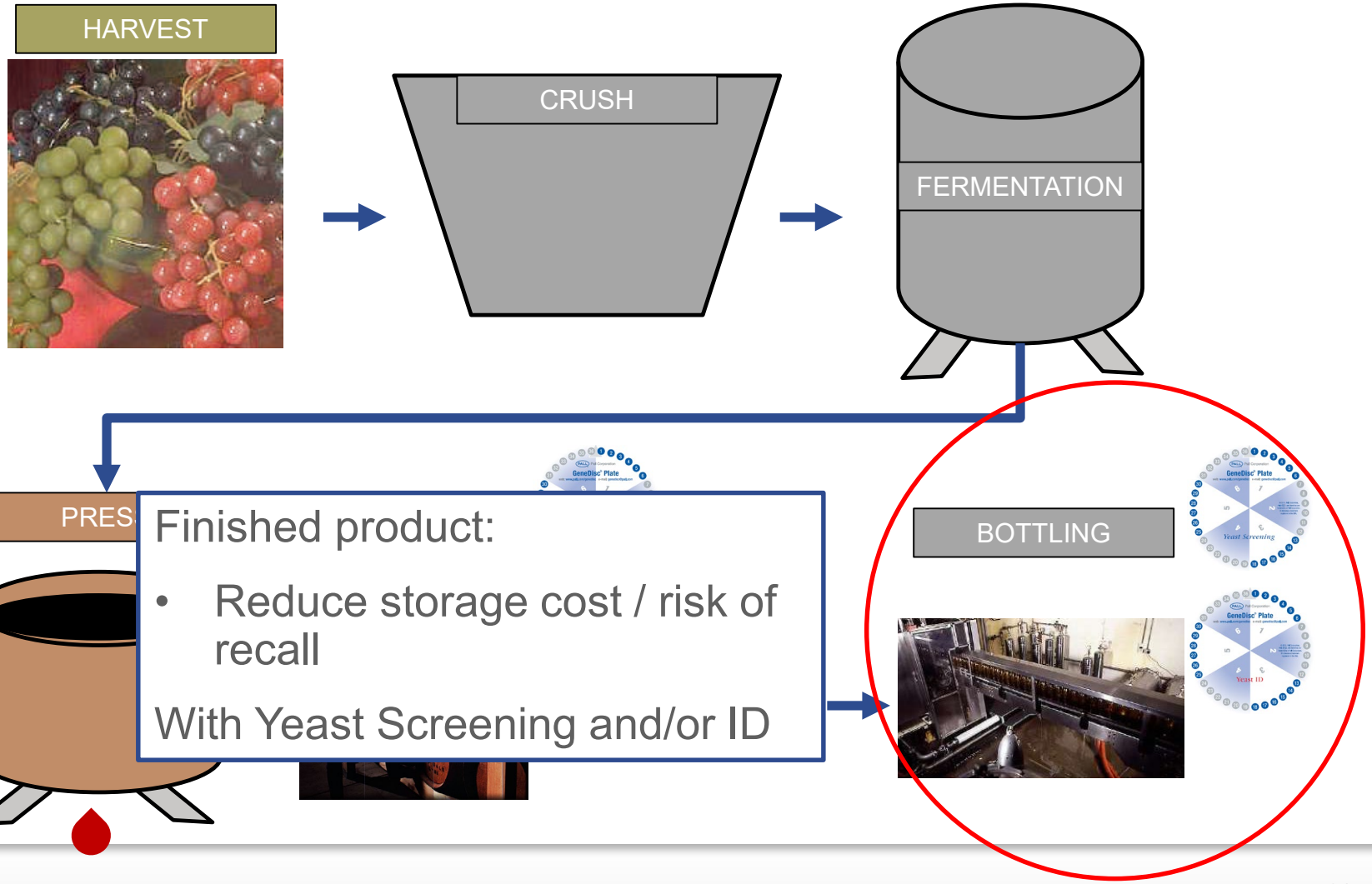
Example of Implementation in Winery



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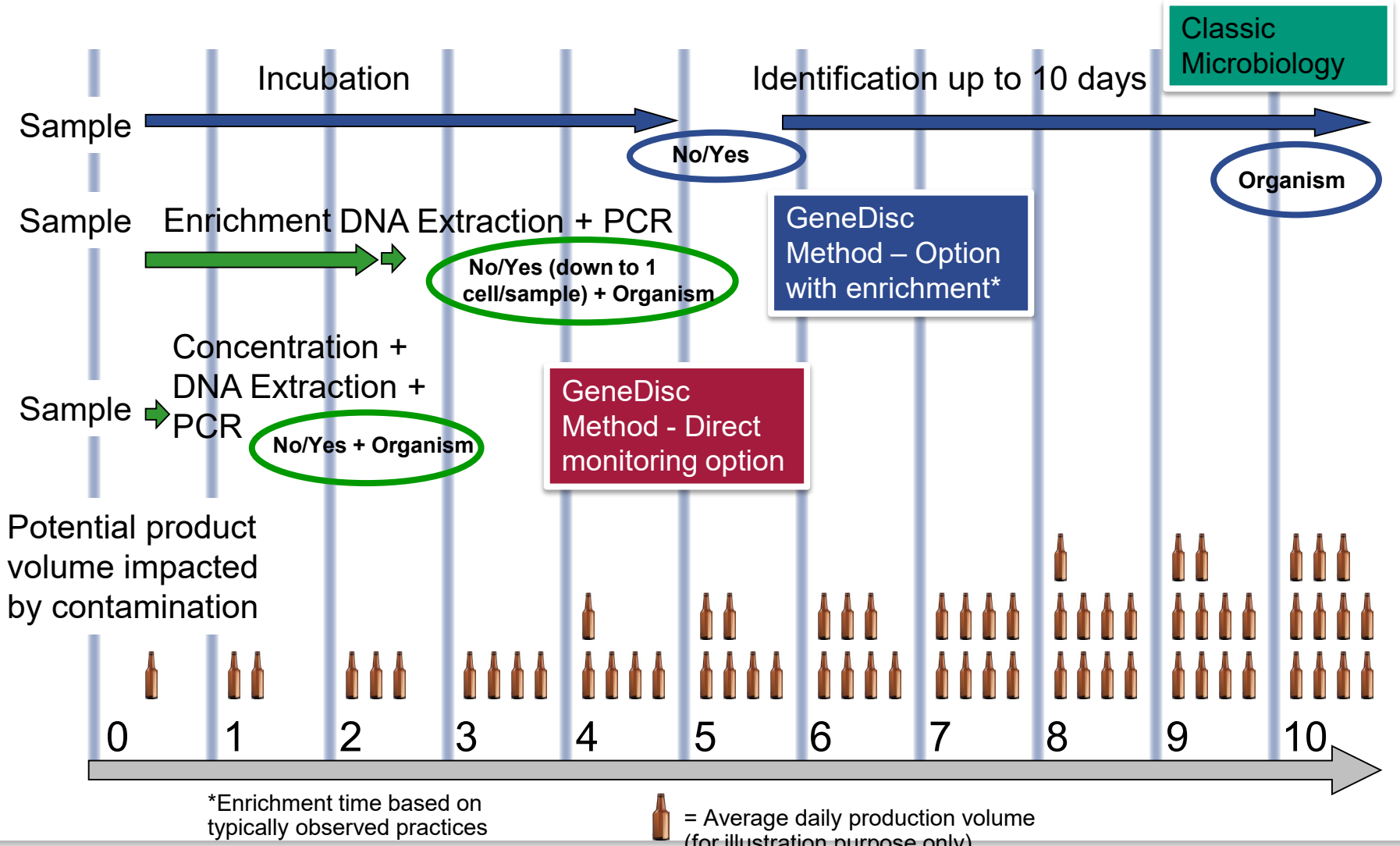
Rapid Microbiology - Early Preventative Controls



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GeneDisc® Solution: The Right Tool for Quality Monitoring Throughout the Wine Making Process

- **Cost-effective and informative method**
  - Can provide answers for the major spoilage organisms in one single run
  - ***Lower analytical cost***
- **Accelerated decision making**
  - Method allows for early preventative controls
  - ***Reduced risk of product spoilage and associated cost***
- **Fast corrective actions implementation**
  - Rapid tracking of contamination cause
  - ***Reduced negative financial impact of spoilage***
- **Adaptable to your priorities**
  - Two GeneDisc plates available (Yeast Screening and Yeast ID)
  - Three testing stategies (Enrichment, direct monitoring and *Brettanomyces* quantification)
  - ***Maximum flexibility***
- **Designed for routine use**
  - Method uses ready to use reagents and results are automatically interpreted
  - ***No microbiology experience is required***

Ensuring Effective Filtration with Integrity Test Devices

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Why Should Integrity Testing be performed?

- **Integrity testing gives assurance of filter performance through a test which is directly linked to the ability of the filter to retain microorganisms**

- **Integrity Testing supports in the following areas:**
  - Brand and financial protection
  - Reduced risk of contamination, product recalls, reprocessing costs, product loss, production delays
  - Regulations requiring monitoring of critical control points and satisfying auditors

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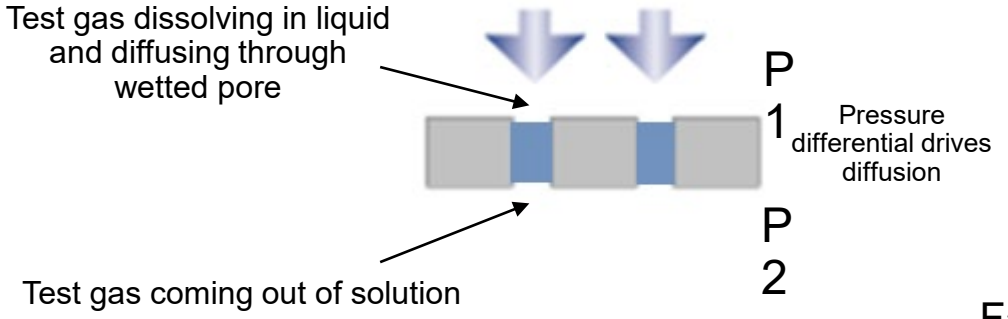
Reduced Risk of Product Contamination, Losses and Recalls

- Pressure decay or forward flow integrity tests directly or indirectly measure the **flow of air through a wetted membrane pore**, when the upstream is held under pressure

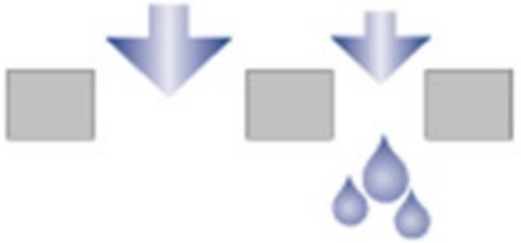
- The flow of air can be a combination of diffusional flow through a wetted pore or bulk flow through an open pore:

Diffusional Flow



Slow, low volume of gas transferred by diffusion through a wetted pore

Bulk Flow

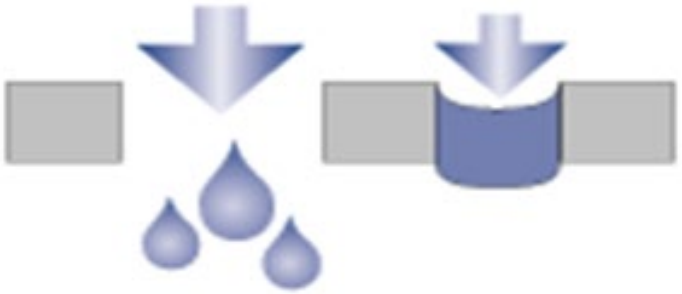


Fast, large volume of gas transferred by free flow through an open pore

Reduced Risk of Product Contamination, Losses and Recalls

- In a defect filter, filter pores are larger due to damage
- The pressure required to expel water from a pore is inversely proportional to the size of the pore

Larger Pore
Less pressure required to expel the wetting fluid



Smaller Pore
Higher pressure required to expel the wetting fluid

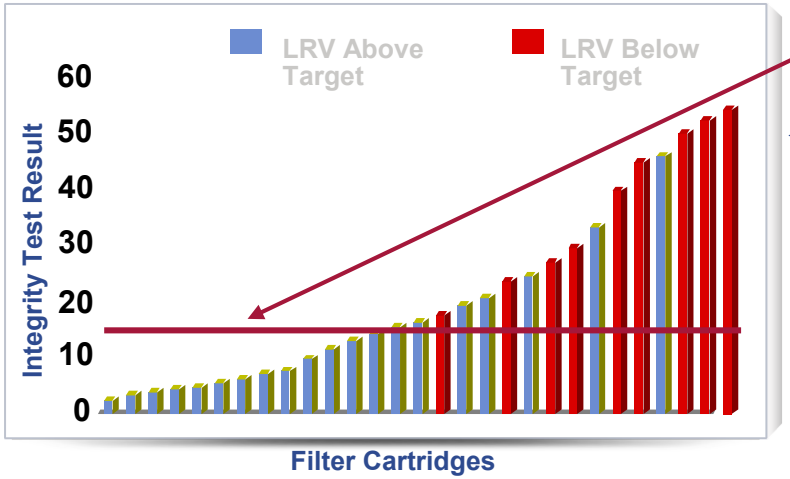
- Therefore the wetting fluid may be expelled from larger, damaged pores at the integrity test pressure, whilst the wetting fluid would remain within the smaller, undamaged pores
- The absence of wetting fluid means bulk flow can take place through these damaged pores or membrane defects
- This gives a higher air flow during the integrity test and therefore a test result exceeding specified limits

- Final membrane filters have an integrity test limit, which customers use to gain assurance of whether their filtration installation is fit for purpose:
  - Result above limit – test fails: installation non-integral and unfit for purpose
  - Result below limit – test passes: installation integral and fit for purpose
- But the integrity test is only measuring air flow / pressure decay
- **How does this correlate to microbial retention?**

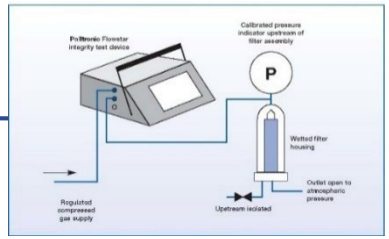
The integrity test limit links true microbial retention (LRV) to an integrity test result

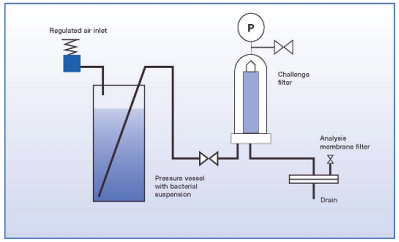
What is LRV (Log Reduction Value) and why is it important?

- LRV is directly linked to the risk of microbial penetration through a filter
- If 1,000,000,000 (10^9) organisms challenge a filter, and 1 penetrates, the LRV can be calculated as 9
- It can also be expressed as a %. Probability that each challenge organism will penetrate this filter = 0.0000001%



Integrity test limit





Integrity testing gives assurance of filter performance to qualified limits based on LRV, protecting the manufacturer's product and brand

- **Pall Integrity Test Parameter Qualification**
  - Goal of an integrity test program – reduce risk and gain assurance of filter performance
  - The ability of an integrity test program to provide its true goal does not just depend on the integrity test device
  - The filter used and the technical standards implemented to qualify integrity test parameters are equally important
  - This gives the end user validated performance against established test limits
  - Pall implement best practice through integrity test parameter qualification to ultimately reduce risk in the end user's process

- **Pall Integrity Test Parameter Qualification**
- Pall Food & Beverage best practice:
  - Safety factors in determination of integrity test limits
  - Multiplication factors when providing integrity test parameters for larger installations – reducing the risk of false pass results (defects hidden in the grey area when testing a large housing)
  - Microorganisms used during challenge testing
    - Culture and growth optimized to ensure uni-cellular distribution (smaller – worse case)
    - Life-cycle growth modelled to use at optimal size

Compact Touch

Protect Your Product. Protect Your Brand.

Filter integrity test device for the Food & Beverage market

Reduced Risk of Product Contamination, Losses and Recalls

Assurance of Filter Performance Through Integrity Testing

Optimized Test Speed and Security

Automatic Purge, Stabilization and Test Function

Pall Integrity Test Parameter Qualification

Ease of Use and Improved Operator Efficiency

Touch Screen Interface

Improved Design

Summary

- Detection, identification and removal of microorganisms is critical for process control and prevention of economic loss

- Two solutions that provide quality control:
  - Rapid detection and/or identification of microorganisms
    - PCR technology - GeneDisc

  - Removal of microorganisms through filtration
    - Ensuring effective filtration through integrity testing – Compact Touch

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Questions?